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Hypothesis

Implications of prognostic variables in the assessment of autoimmunity in hepatitis C patients receiving interferon therapy

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Abstract:
Systematic administration of interferon-alpha (INF-alpha) is considered as the backbone of HCV therapy since 1991. Interferon (IFN) therapy can cause vasculitis, glomerulonephritis, cryoglobulinemia and certain other autoimmune diseases such as sialoadentitis, lichen planus and thyroiditis. Related to the factors of interferons, extensively studied gland is thyroid gland. A strong association was observed between thyroid disease and HCV patient when they were exposed to IFN therapy. Vitamin D, malondialdehyde (MDA), thyroid hormones and auto antibodies were biochemically assessed from the venous blood of seventy five HCV patients and fifty healthy controls. The results of all parameters were analyzed by independent sample t-test. The results of the study demonstrated a clear picture that the levels of vitamin D decreased as compared to control but increases in case of MDA. The levels of antibody titer represent that thyroglobulin-antibody (TGAb) thyroid peroxidase-antibody (TPOAb) as well as thyroid stimulating hormone receptor-antibody (TSHRAb) were raised in the patients suffering from HCV with thyroid dysfunction as compared to control. Similarly, the levels of thyroid hormones were also elevated in the HCV patients. Antibodies generated against thyroidal enzymes leads to impaired function of these enzymes thus causing decreased synthesis of thyroid hormones. As exogenous INF triggers the release of cytokines that mediate the recruitment of immune cells with increased production of inflammatory markers lead to production of lytic granules which have direct toxic action on thyroid cells and ultimately increased lipid peroxidation of thyrocytes. The results of the present study clearly demonstrated that the decreased levels of vitamin D in HCV patients receiving IFN therapy were responsible to induce autoimmunity against thyroid gland and adjutant therapy may be helpful to alleviate the possible thyroid disorders.

Key words: Interferon alpha; Hepatitis C virus; Vitamin-D; Interleukin-6; Tumor necrosis factor alpha

Background:
Hepatitis C is the major reason for the chronic liver disease and hepatocellular carcinoma affecting 150-200 million people worldwide. Approximately 3 percent of the world population is infected with Hepatitis C Virus (HCV) causing about 500,000 deaths per annum. Rapid production and inadequate proofreading

by viral RNA polymerase results in several mutant variants of HCV due to which it is a challenge for researchers to develop vaccines against this virus. So far six genotypes have been identified and are further classified into subtypes (a, b, c, d, etc.) **[1].** The principal mode of transmission of HCV is through blood transfusions, intravenous drug use and unsterilized equipment. The most efficient and well-known therapy for HCV is the administration of ribavirin and interferon alpha (IFN-alpha) as a combination. A major and frequent adverse effect of this treatment is the development of thyroid disease.

A large variety of autoimmune thyroid diseases (TD) has been reported ranging from Graves' disease to Hashimoto's thyroiditis and destructive thyroiditis **[2].** Encountered in about 11,241 consecutive patients who were going through the disease condition and especially they were offered with interferon therapy, out of which very less almost 71 which accounts for (0.6%) have developed symptomatic thyroid disorders **[3].** The set of study however, is like estimation as there was no repeated surveillance in functions of thyroid gland therefore symptoms of thyroid disorders can easily be misjudged. Studies in such with repeated thyroid functions checked have developed thyroid dysfunction in about 4-14% of patient population who were subjected to interferon alpha therapy **[4].**

Materials and Methods

Patients

All the selected patients were screened at the Teaching Hospital, University College of Medicine and Dentistry, the University of Lahore. Seventy five hepatitis C patients in the age group of 25-70 years were eligible for inclusion in the study. Informed written consent was obtained before being included in this study. Fifty age and sex-matched clinically healthy individuals were included as controls. The research ethical committee of University of Lahore approved the experimental protocol. The diagnosis of HCV was based on the following criteria: 1) Increased serum levels of amino-transferases for at least six months; 2) Absence of anti-thyroid antibodies before the start of interferon therapy. The patients who complete the interferon therapy with the presence of anti-thyroid antibodies were selected for this study. The subjects with the history of taking drugs (including alcohol and cigarette), pre-diagnosis medications (e.g. antiparkinsonian/antipsychotic), were excluded from this study. Sera were separated by centrifugation for 10 minutes at 3000 rpm and stored at -80 ^{o}C until biochemical analysis.

Biochemical analyses

Lipid peroxidation was estimated calorimetrically as previously described **[5].** 200µl sample was taken in test tube then 200µl of 8.1% SDS, 1.5ml of acetic acid (20%) and 1.5ml of TBA (0.8%) were also added in test tube and heated for 60min. After cooling, 4ml of n-butanol was added and was centrifuged for 10min at 3000rpm. The upper organic layer was separated and absorbance was taken at 532nm against the blank. T3 (Triiodothyronine), T4 (Thyroxine), TSH (Thyroid stimulating hormone), TSHr and TPO (Thyroid peroxidase) IgG were estimated by using human ELISA kit (BioVendor). Vitamin-D was determined by the ELISA kit method of ALPCO, USA. (Heaney RP2010) **[6].**

Results:

The data demonstrated that the levels (8.34±1.11nmol/ml) of MDA in HCV patients increased significantly as compared to the healthy controls (1.34±0.02, p=.027). The levels of IL-6 (7.87±2.76, p=.019), TNF-α (35.76±3.99, p=.039), and vitamin D (11.87±2.76, p=.011) were also significantly differed as compared to control (3.87±.021), TNF-α (21.34±3.67), and vitamin D (21.65±4.7) respectively. The levels of antibody titer represent that thyroglobulin-antibody (TGAb) (54%), thyroid peroxidase-antibody (TPOAb) (48.35%) as well as thyroid stimulating hormone receptor-antibody (TSHRAb) (60.85%) were raised in the patients suffering from hepatitis C with thyroid dysfunction as compared to control (26.55±6.27, 5.19±1.31 and 1.19±0.17 respectively). Similarly, the levels of thyroid hormones like FT4 (Free T4) (50.65%), TSH (64.20%) and rT3 (Reverse T3) were also elevated in the patients suffering from hepatitis C with thyroid dysfunction as compared to control (10.53±1.40, 1.84±0.84 and 19.36±3.28 respectively).

Discussion:

The present study revealed that thyroid antibodies titer was raised in the patients suffering from hepatitis C along with thyroid dysfunction. The levels of vitamin D were also decreased and had an inverse relation to TSHRAb (Vit-D vs TSHRAb: r= -.438**). Likewise, the levels of thyroid hormones like FT4 (Free T4), TSH and rT3 (Reverse T3) were elevated in the patients having hepatitis C and thyroid dysfunction simultaneously. In vitro studies of vitamin D have provided idea about immunomodulatory effect of thyroid cells such as Th1, Th2, activated T cells and dendritic cells. Vitamin D supplementation has reduced the risk of many autoimmune disorders such as thyroid dysfunction in HCV patients. A high prevalence of thyroid autoimmunity is observed in patients using thyroid microsomal and thyroid peroxidase autoantibodies **[7].** The molecular mechanism was investigated to reveal the immunomodulatory function of vitamin D in autoimmune thyroiditis mediated by harboring vitamin D3 receptor (VDR). The immunomodulatory properties of vitamin D were associated with the activation of T and B lymphocytes. T-cell activation which is dependent upon dendritic cells is inhibited by vitamin D. A current in vitro study also made clear the molecular mechanism by explaining the expression of vitamin D receptor through the activation of CD4 T cells promoting the production of IL-4 and IL-5 along with Th2 phenotype. Although, the Th1 is suppressed due to the presence of interferon-gamma (IFN-gamma) in the cell. Some data regarding lower level of vitamin D in autoimmune thyroiditis explains that there might be some mal-absorption e.g., systemic sclerosis, reduced sun exposure or photosensitivity e.g., SLE and dermatomyositis. Another possible mechanism suggests that there might be an increase in bone turnover of patients with hyperthyroidism causing high calcium

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levels and generates a negative feedback on parathyroid hormone and vitamin D3 synthesis. Several thyroid autoimmune disorders are linked with allelic variations of VDR gene especially AITD is associated with the polymorphism of VDR **[8].** This association is demonstrated with the correlation between F allele's carriers of VDR-folk-I polymorphism and Graves' disease. Therefore, vitamin D replacement therapy might be beneficial in AITD patients **[9].**

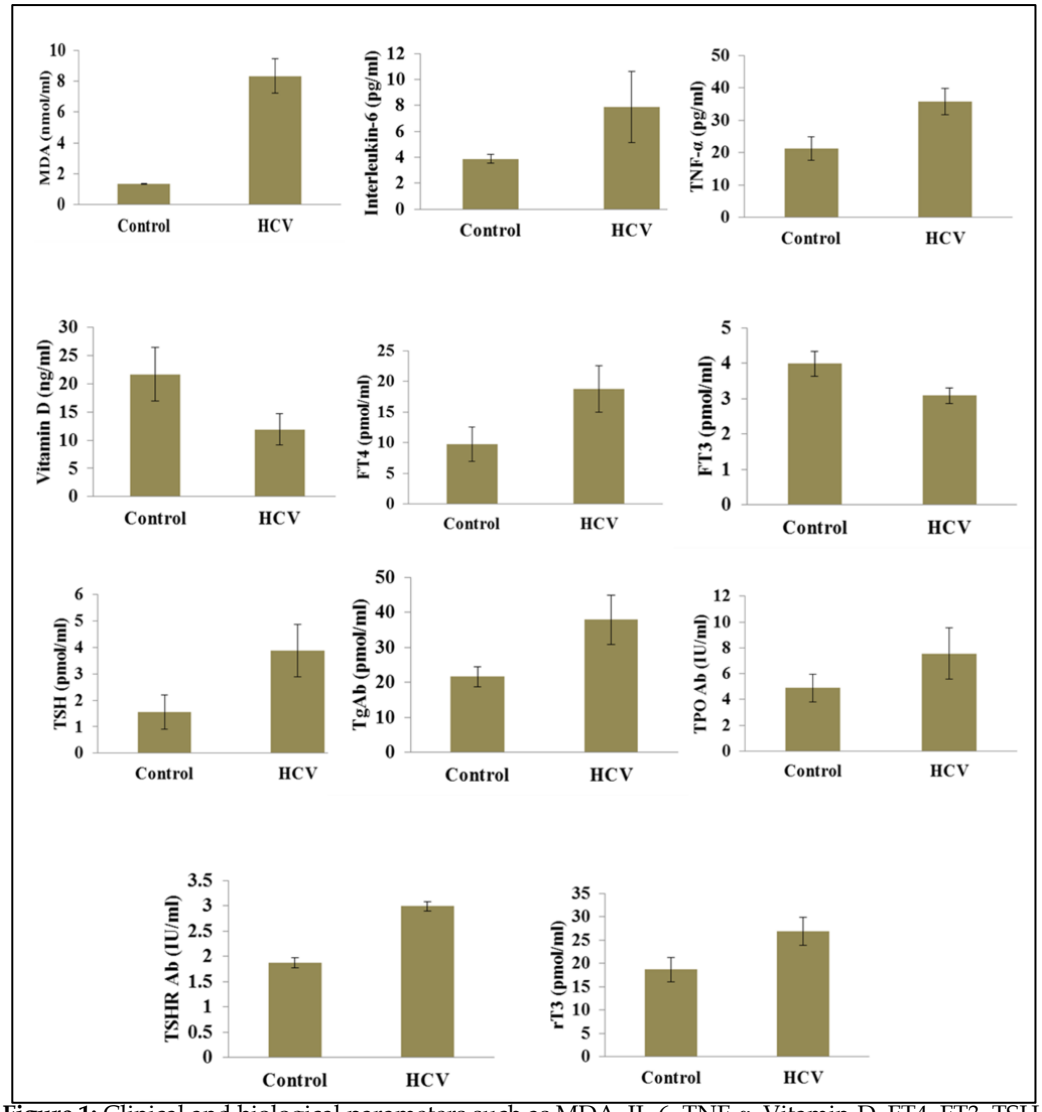

Figure 1: Clinical and biological parameters such as MDA, IL-6, TNF-α, Vitamin-D, FT4, FT3, TSH, TgAb, TPO Ab, TSHR Ab and rT3 were analyzed in healthy controls and HCV patients treated with interferon.

It has been suggested that inhibition of proliferation of T helper cells especially Th1, a balancing situation is created by increasing the production of Th2 lymphocytes which are responsible for the increased production of several cytokines especially IL-6 which is inversely related to the vitamin D activity (IL-6 vs vitamin D: r= -.416*). IL-6 levels correlated positively with IL-2 and TNF-alpha (TNF-α vs IL-6: r= .379*) that augments the effects of IL-2 thus promoting the differentiation of Th1 and Th2 cells **[10].** In the present study, vitamin D and TNF-alpha have a negative relation (TNF-α vs Vit-D: r= -.791**) and was reported previously by Barchetta *et al.* (2012) **[11].** Other studies showed that the vitamin D inhibited the replication of HCV and the sustained virological responses (SVR) can be improved with vitamin D supplementation. Thus, it has been proposed that MDA and vitamin D were negatively correlated as shown (MDA vs Vit-D: r= -.853***). As long as vitamin D deficiency is concerned, it is present in about one third

of the population diagnosed with the liver diseases. It is evident that the active form of vitamin D work synergistically with IFN, thus appropriate amount of vitamin D will be required for proper therapeutic effect of IFN in HCV patients.

Conclusion:

As exogenous INF act as co-stimuli and triggers the release of cytokines that mediate the recruitment of immune cells with increased production of inflammatory molecules that augment the production of lytic granules which have direct toxic effect on thyroid cells and ultimately increased lipid peroxidation. In conclusion, the higher levels of vitamin D in HCV patients receiving IFN therapy may aid in the alleviation of IFN induced autoimmune thyroid disorders and serves as a diagnostic factor with therapeutic significance.

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Conflict of Interest:

Authors declare that there is no conflict of interest.

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